

# **Engineered Antibodies for the Treatment of Anthrax**

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the Institute for Cellular and Molecular Biology  
University of Texas, Austin TX 78712**

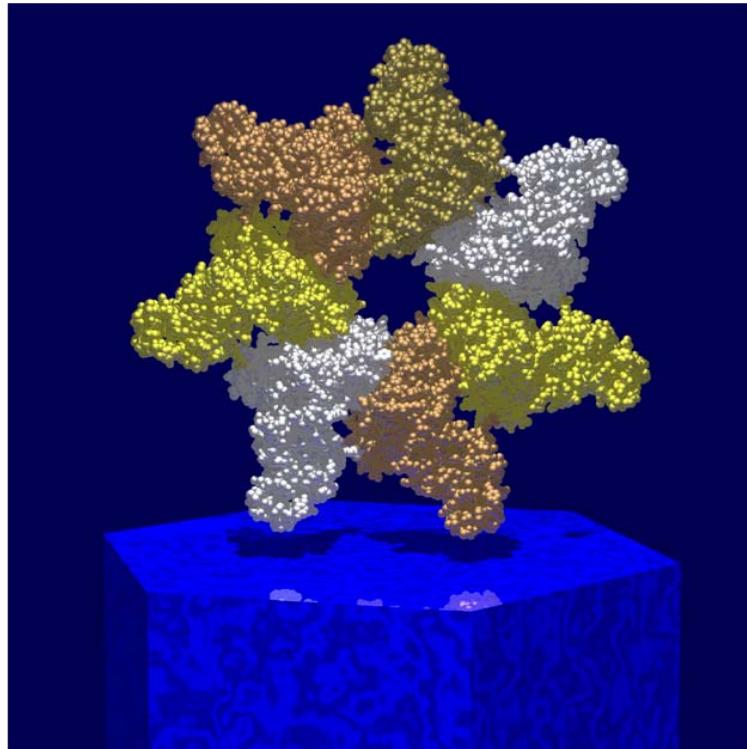
## Passive Protection by Polyclonal Antibodies against *Bacillus anthracis* Infection in Guinea Pigs

S. F. LITTLE,\* B. E. IVINS, P. F. FELLOWS, AND A. M. FRIEDLANDER

*Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases,  
Fort Detrick, Maryland 21702-5011*

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The protective effects of polyclonal antisera produced by injecting guinea pigs with protective antigen (PA), the chemical anthrax vaccine AVA, or Sterne spore vaccine, as well as those of toxin-neutralizing monoclonal antibodies (MAbs) produced against PA, lethal factor, and edema factor, were examined in animals infected with *Bacillus anthracis* spores. Only the anti-PA polyclonal serum significantly protected the guinea pigs from death, with 67% of infected animals surviving. Although none of the MAbs was protective, one PA MAb caused a significant delay in time to death. Our findings demonstrate that antibodies produced against only PA can provide passive protection against anthrax infection in guinea pigs.



Antibodies to the PA toxin could serve two roles:

1. As a prophylactic to prevent spore germination in analogy to the vaccine (passive immunization)
2. As a late stage antitoxin to serve as an antidote past the point at which antibiotic therapy is effective

# First Generation Enhanced 14B7

<i>Antibody Variant</i>	$k_{\text{on}} (*10^5 \text{ M}^{-1} \text{ sec}^{-1})$	$k_{\text{off}} (*10^{-4} \text{ sec}^{-1})$	$K_d (\text{nM})$
14B7 scAb	$2.8 \pm 0.3$	$30 \pm 0.8$	12
1H scAb	$6.1 \pm 0.9$	$1.6 \pm 0.4$	0.26

# Lethal Toxin Challenge



**Animal Model --> Rat**

**Antidote --> Inject 4x and 1.5x Excess of Antibody 5 Minutes Prior to Toxin**

**Challenge --> Venous Injection of Toxin (10X Lethal Dose)**

# Lethal Toxin Challenge

<i>Treatment</i>	$K_d$ (nM)	<i>TTD</i> (min) *	<i>Survivors</i>
PBS	-	82,87,92,97,99	0/5
L97 scFv	63	64,66,67,70,77	0/5
14B7 scFv	12	85,103,112,123,130	0/5
A2E scFv	4	171,242,271	2/5
1H scFv	0.25	212,238	3/5
14B7 scAb	12	102,115,140,172,292	0/5
1H scAb	0.25	--	5/5
1H scAb, (1.5X concentration)	0.25	152	4/5

\*Total time of experiment 5 hrs

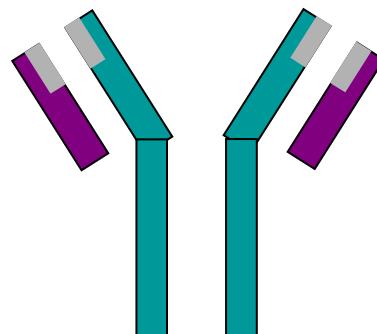
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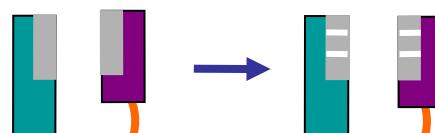
\*Total time of experiment 5 hrs

# Engineering of Anthim™-NIH Funded

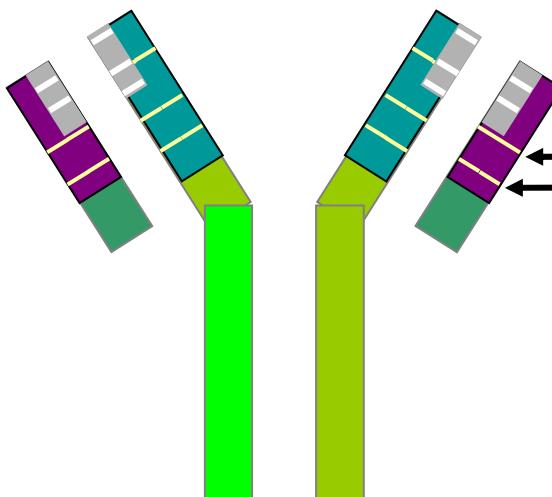
**14B7 (USAMRIID/NIH)**



**ScFv 1H (UT)**



Affinity Enhancing Mutations



Delmmunization  
Mutations

Little S.F., S. H. Leppla, and  
E. Cora. 1988. *Infect Immun.*  
56:1807-13.

Maynard J.A., C.B. Maassen,  
S. H. Leppla, et al. 2002.  
*Nat Biotechnol.* 20:597-601

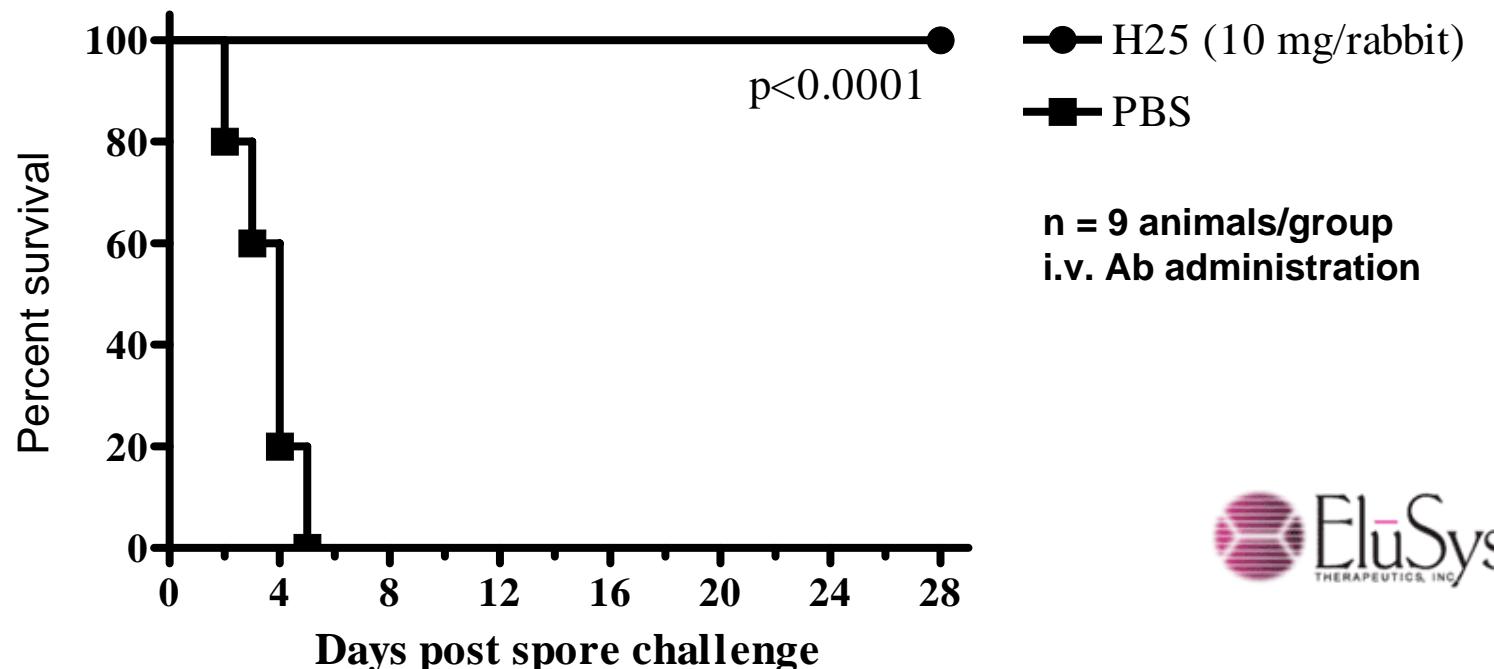


**Anthim™ (H25)**

# Spore Challenge

## Can the antibody act as a prophylactic treatment?

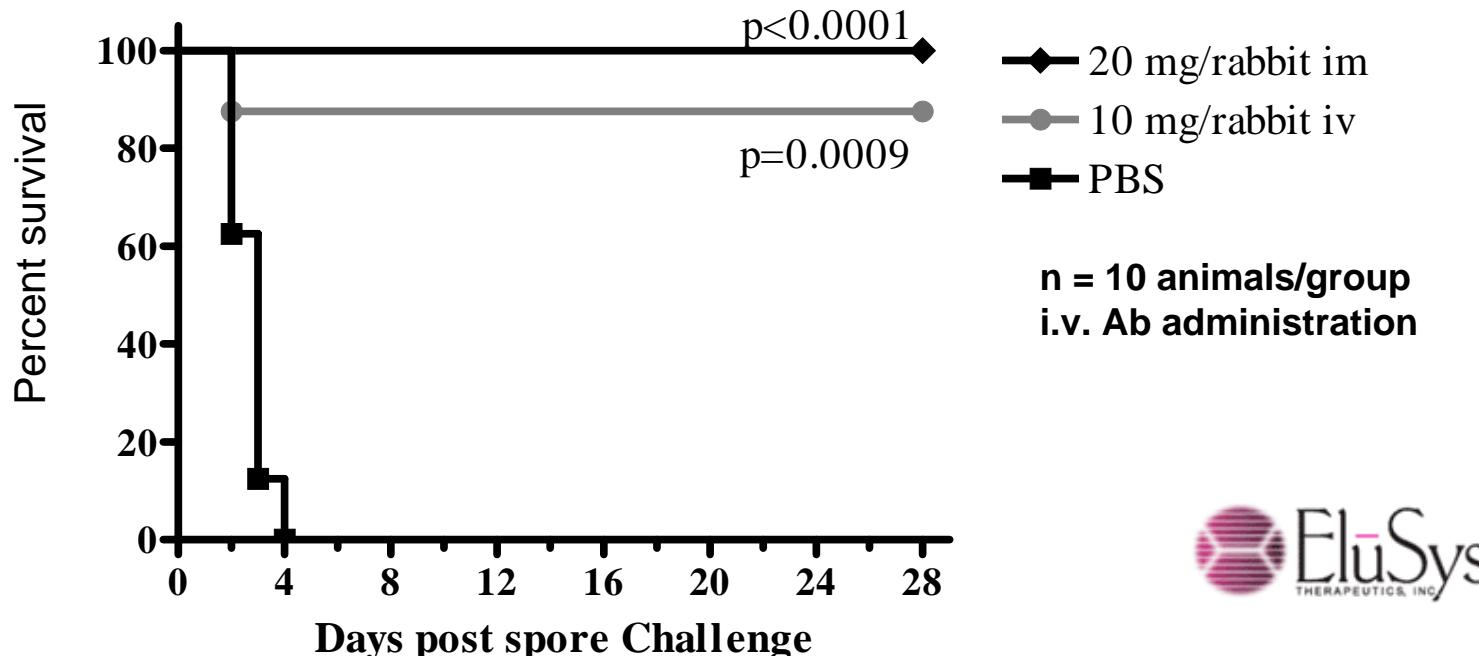
Rabbit Model 100-300 LD<sub>50</sub>'s Aerosol Challenge (Ames)



# Spore Challenge

## Can the antibody act as a prophylactic treatment?

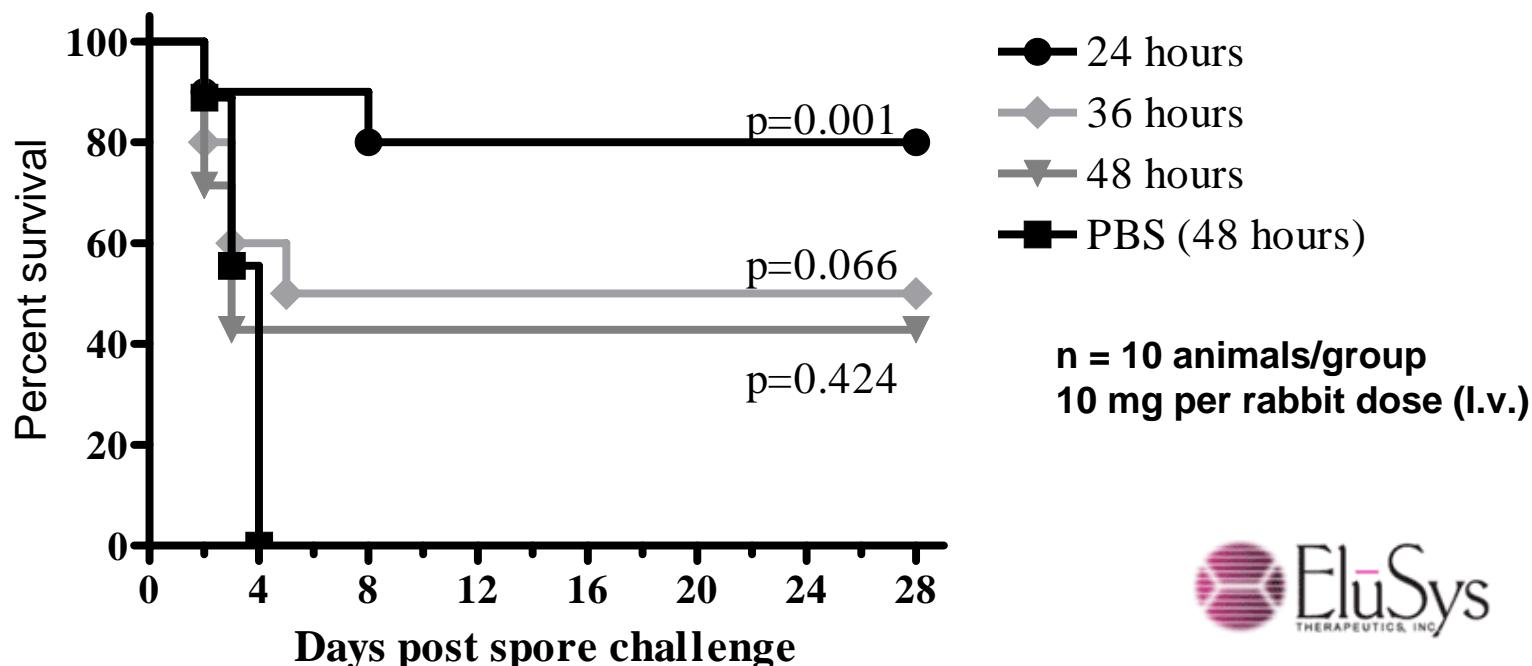
### Rabbit Model 100-300 LD<sub>50</sub>'s Aerosol Challenge (Ames)



# Spore Challenge

Can the antibody act as a post-exposure treatment?

## Rabbit Model 100-300 LD<sub>50</sub>'s Aerosol Challenge (Ames)



# Anthim™ Treated Rabbits Are Free of Bacilli in Blood, Spleen

Animals positive for bacteria in blood								
Group	day1	day2	day7	day10	day14	day21	day28	At Death
Anthim™	0/9	0/9	0/9	0/9	0/9	0/9	0/9	--
PBS	0/5	3/5						4/5
Animals positive for bacteria in organs day 28								
Group/Organ	Lung		Lung-assoc. nodes			Spleen		
Anthim™	2/9		1/9			0/9		

# Anthim™ Treated Mice Are Free of Bacilli in Spleen

Intratracheal challenge, 1xLD100 Sterne in DBA mice (CR Lyons, UNM)

Mouse ID	CFU/ Lung (Day 15)	CFU/ Spleen (Day 15)
14B7, 1	<b>19.6 X 10<sup>4</sup></b>	<b>bd*</b>
14B7, 2	<b>13.5 X 10<sup>4</sup></b>	<b>bd</b>
14B7, 3	<b>10.8 X 10<sup>4</sup></b>	<b>0.04 X 10<sup>4</sup></b>
Anthim™, 1	<b>1.7 X 10<sup>4</sup></b>	<b>bd</b>
Anthim™, 2	<b>13.4 X 10<sup>4</sup></b>	<b>bd</b>
Anthim™, 3	<b>10.5 X 10<sup>4</sup></b>	<b>bd</b>
Anthim™, 4	<b>1.2 X 10<sup>4</sup></b>	<b>bd</b>
Anthim™, 5	<b>1.1 X 10<sup>4</sup></b>	<b>bd</b>

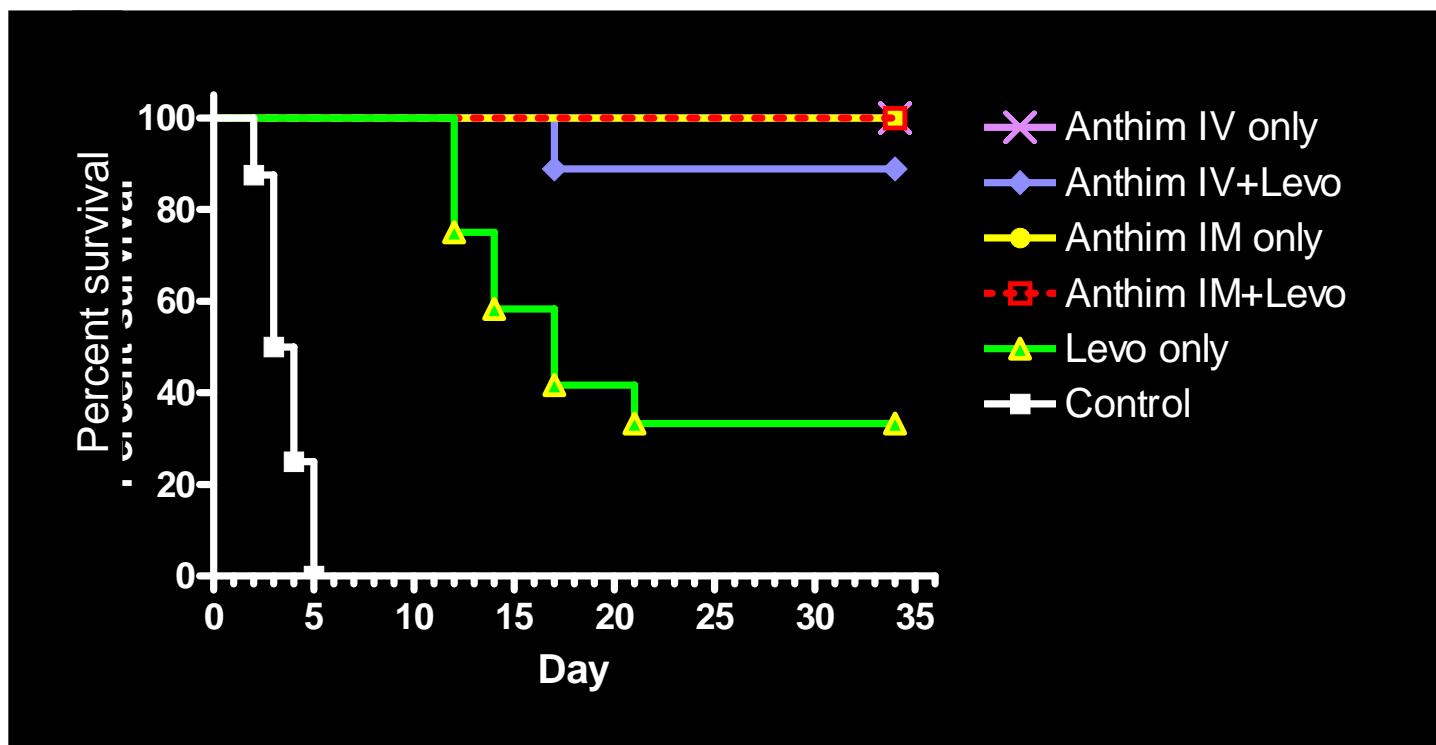
Control mice all died.

\*bd = below detection



# AR007: Rabbit Spore Challenge +/- Fluoroquinolone

**Objective:** To demonstrate that post-exposure administration of Anthim™ by the IV or IM route leads to increased survival above that of levofloxacin after spore challenge



# Clinical Evaluation of Anthim™

## AH-101: Dose-Escalation Phase 1

- Safety, tolerability, PK of single IV dose of Anthim (ETI-204).  
Screen for interaction with ciprofloxacin (cipro)
  - Randomized, placebo-controlled
  - double blind

### Part 1: Single dose Anthim infused IV over 90 min. at 3 dose levels

1. 19 mg; 6 Anthim, 2 PBO, M/F
2. 57 mg; 6 Anthim, 2 PBO, M/F
3. 114 mg; 6 Anthim, 2 PBO, M/F

### Part 2: Single dose Anthim infused IV over 90 min. at highest dose level +/- 500 mg BID cipro for 14 days, 6 subjects/group

1. 114 mg; Anthim + cipro
2. Control; PBO + cipro

# Clinical Evaluation of Anthim™

## AH-101: Dose-Escalation Phase 1

- Safety, tolerability, PK of single IV dose of Anthim (ETI-204).  
Screen for interaction with ciprofloxacin (cipro)
  - Randomized, placebo-controlled
  - double blind

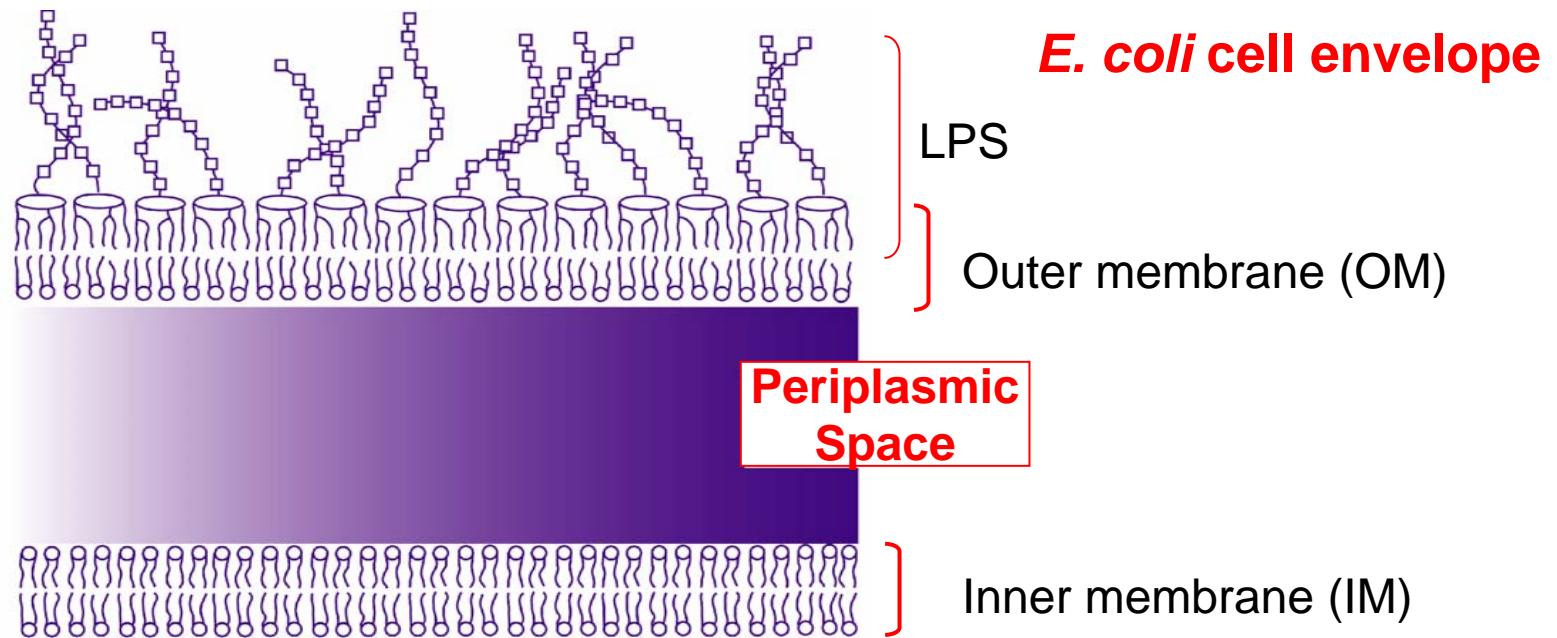
## **Study ongoing**

- **No serious adverse events**
- **No injection site reactions**

## **Key Features of *E. coli* that Accelerate Protein Engineering**

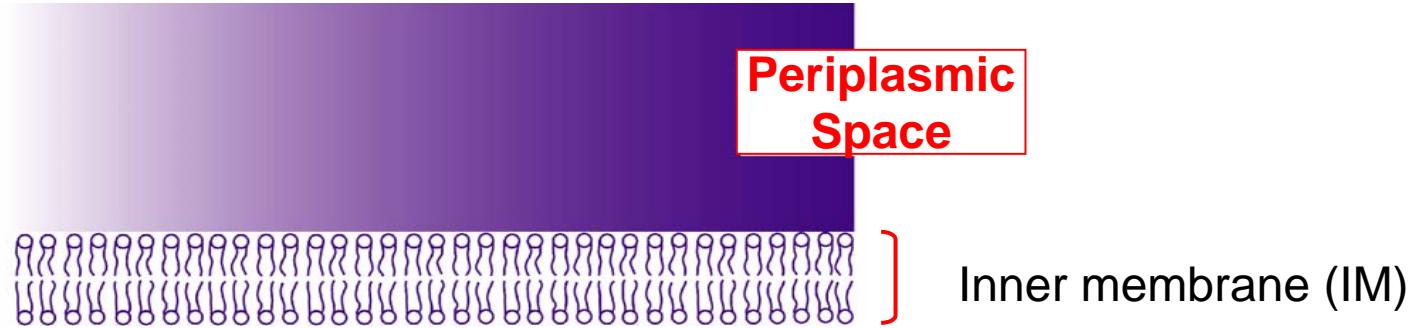
- High transformation efficiency for library production
- Fast doubling time facilitating routine manipulations
- Many specialized strains and tools are available for cloning and protein expression
- Amenable to quantitative FACS sorting ( $10^7$  clones per hour)

## Key Features of *E. coli* that Accelerate Protein Engineering



- Dual membrane structure with periplasmic space that can facilitate interactions between co-expressed proteins

## Key Features of *E. coli* that Accelerate Protein Engineering



- Dual membrane structure with periplasmic space that can facilitate interactions between co-expressed proteins
- Outer membrane can be selectively permeabilized with detergent or largely removed by spheroplasting

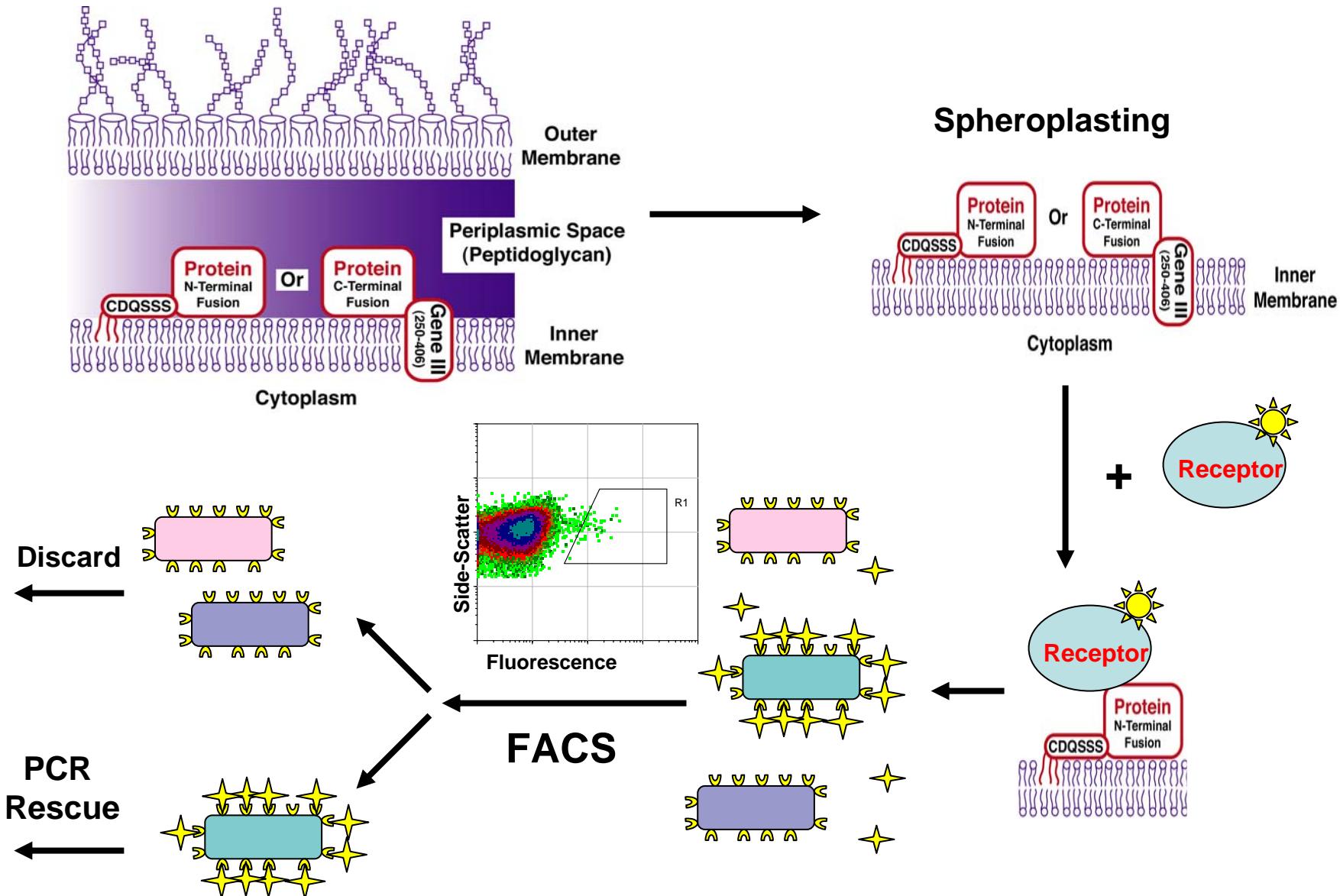
## Anchored Periplasmic Expression (APEx)

- Isolation of ligand binding proteins ✓✓✓✓ (similar to yeast display)
- Protein:protein interactions ✓✓✓✓ (quantitative)
- Expression maturation ✓✓
- *Enzyme engineering* yes, for hydrolytic enzymes
- *Suitable for the engineering of multi-subunit proteins & proteins with complex cofactors*
- *Membrane protein engineering*

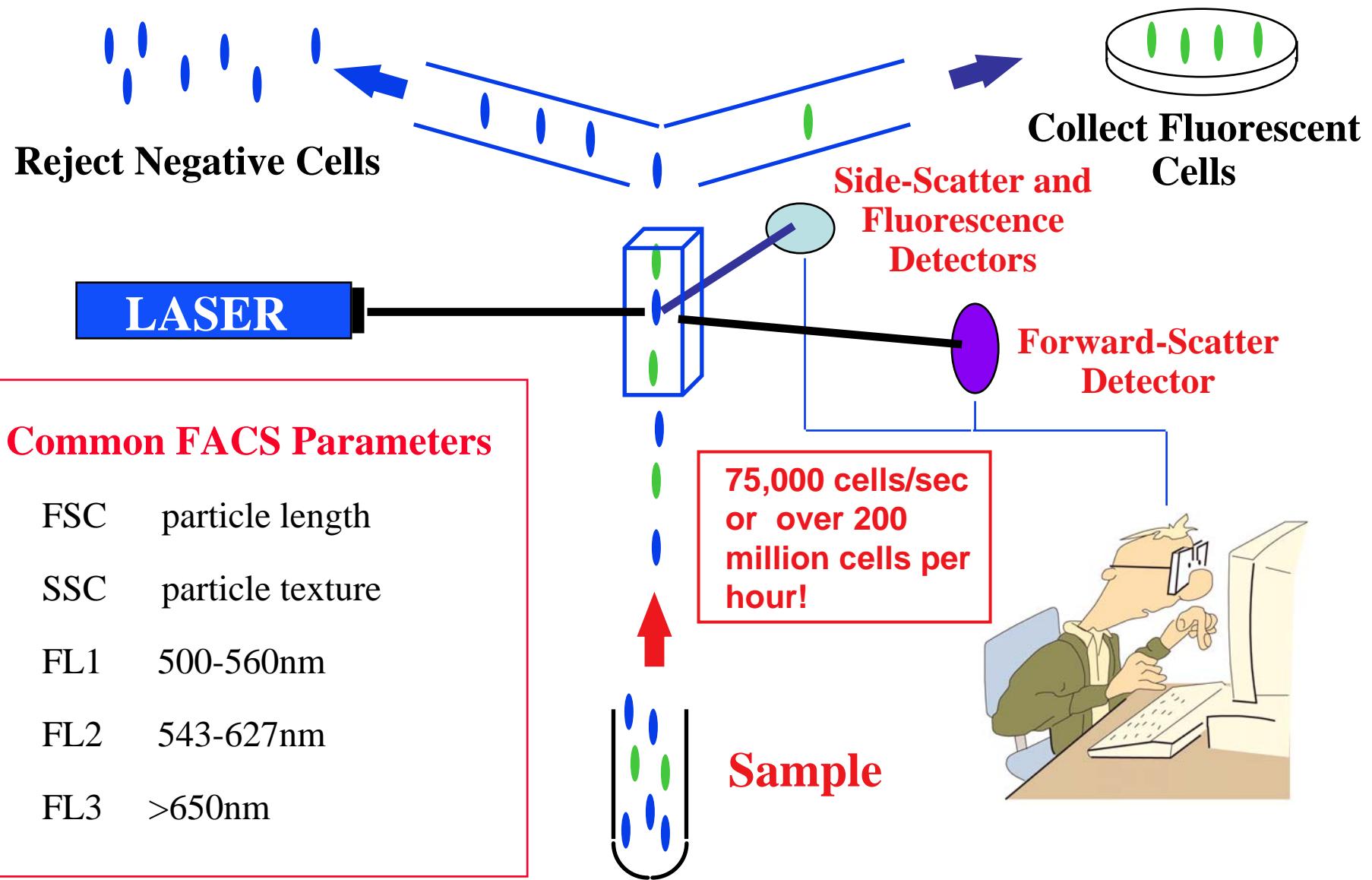
### Commercial considerations

- Available; licensed by Merck, Pfizer, Lilly, and others

# Anchored Periplasmic Expression (APEx)

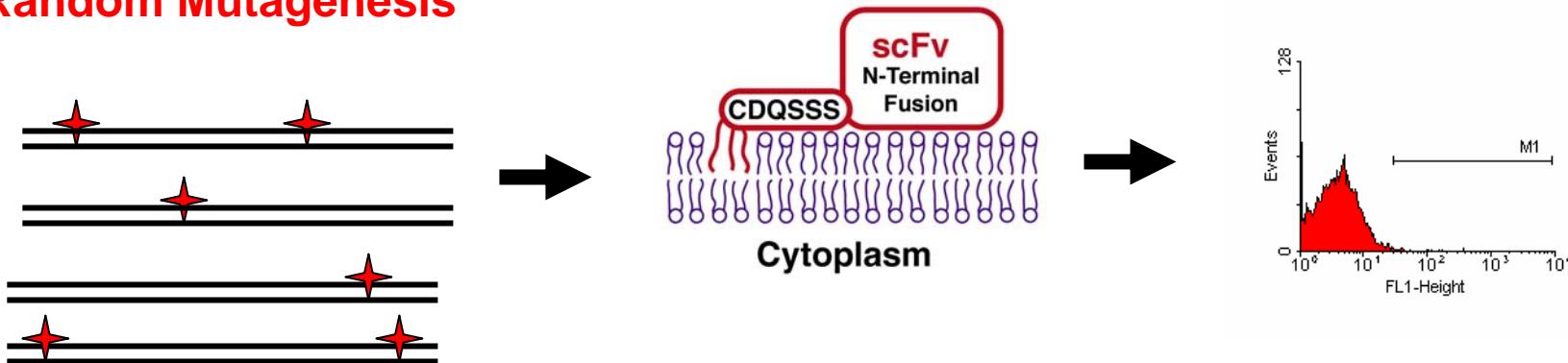


# Fluorescent Activated Cell Sorting (FACS)

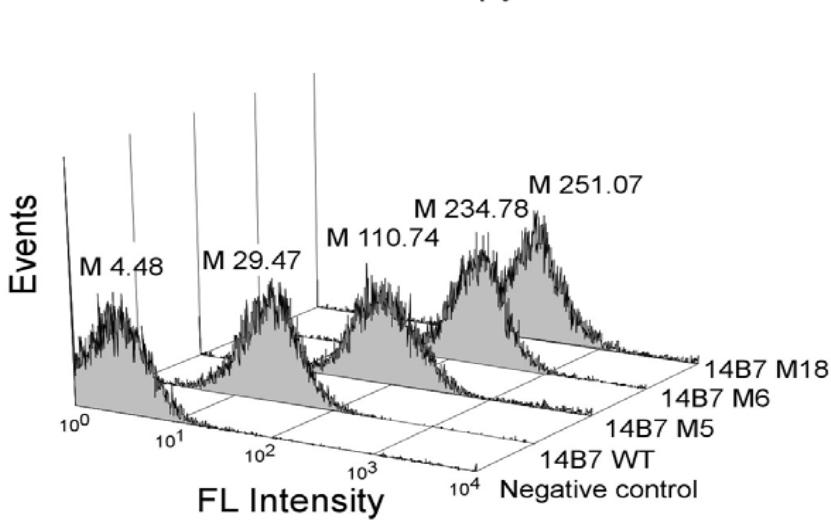


## Isolation of High Affinity Anti-PA Antibodies

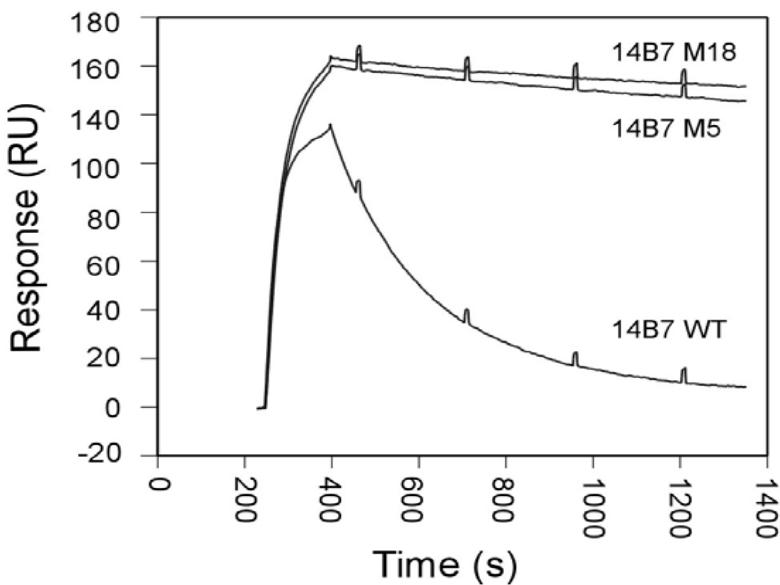
### Random Mutagenesis



A Flow Cytometry Scans  
200nM PA-BodipyFL



B BIACore Results



## Ultra-High Affinity PA Neutralizing Antibodies

<i>Antibody Fragment</i>	$k_{\text{on}} (*10^5 \text{ M}^{-1} \text{ sec}^{-1})$	$k_{\text{off}} (*10^{-4} \text{ sec}^{-1})$	$K_D (\text{nM})$
14B7	$7.1 \pm 0.3$	$30 \pm 0.8$	4.3
1H	$6.4 \pm 0.8$	$1.7 \pm 0.3$	0.25
M18.1	$11 \pm 4$	$0.24 \pm 0.03$	0.021
<b>M18.1 Optimized</b>	<b><math>8.3 \pm 2</math></b>	<b><math>0.10 \pm 0.02</math></b>	<b>0.012</b>



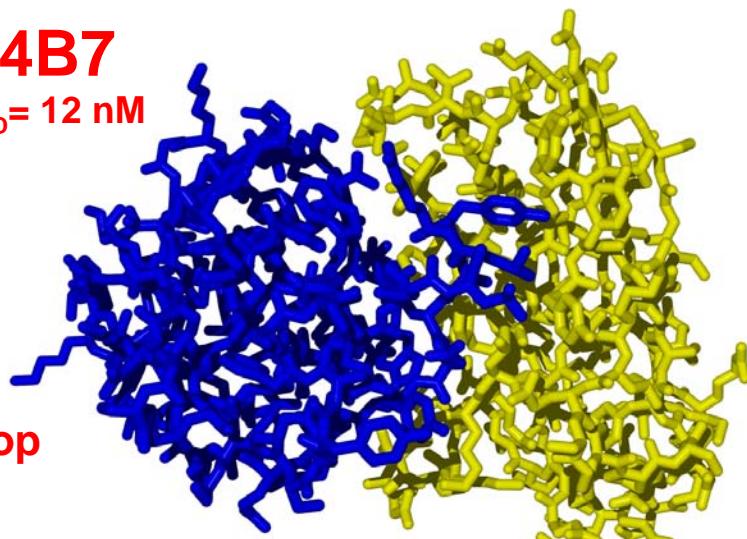
$t_{1/2} \sim 20 \text{ hours}$

# X-tal Structures: Before and After APEx

**14B7**

$K_D = 12 \text{ nM}$

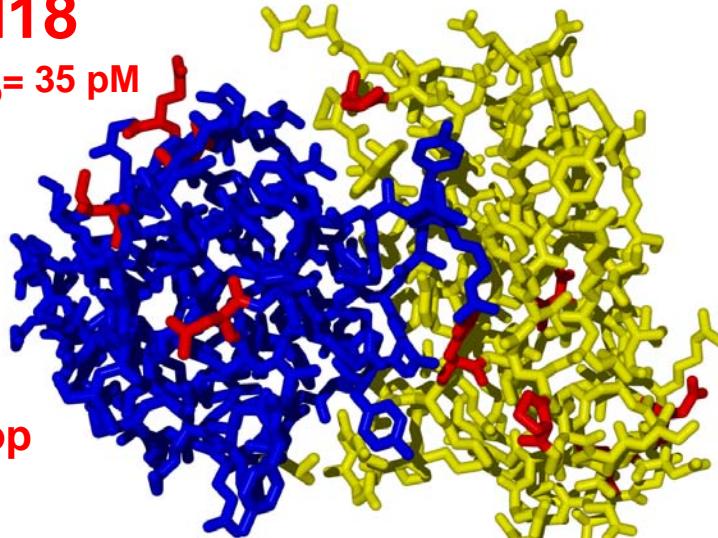
Top



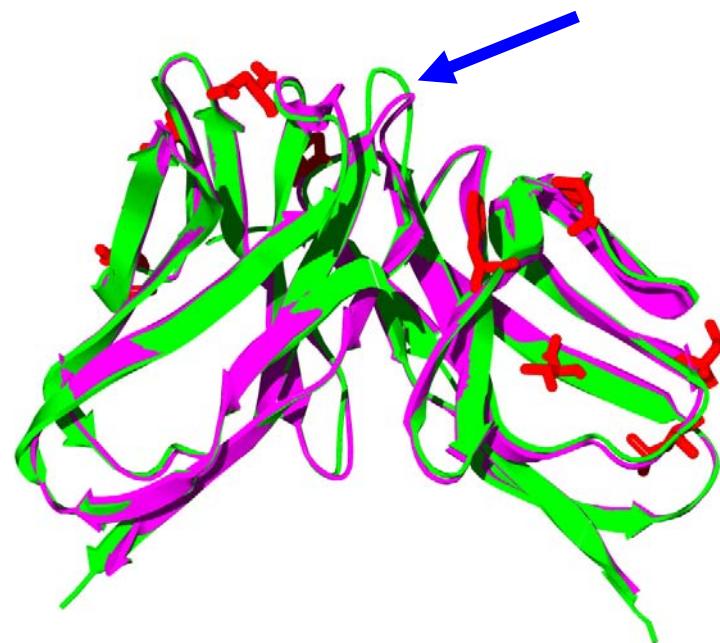
**M18**

$K_D = 35 \text{ pM}$

Top

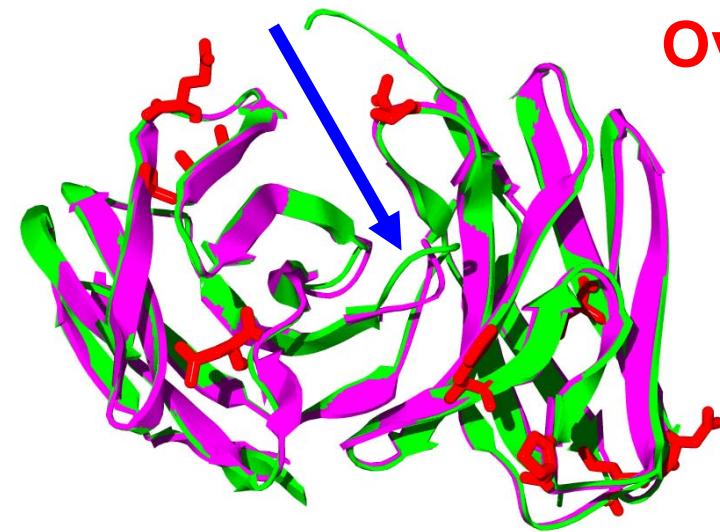


Side



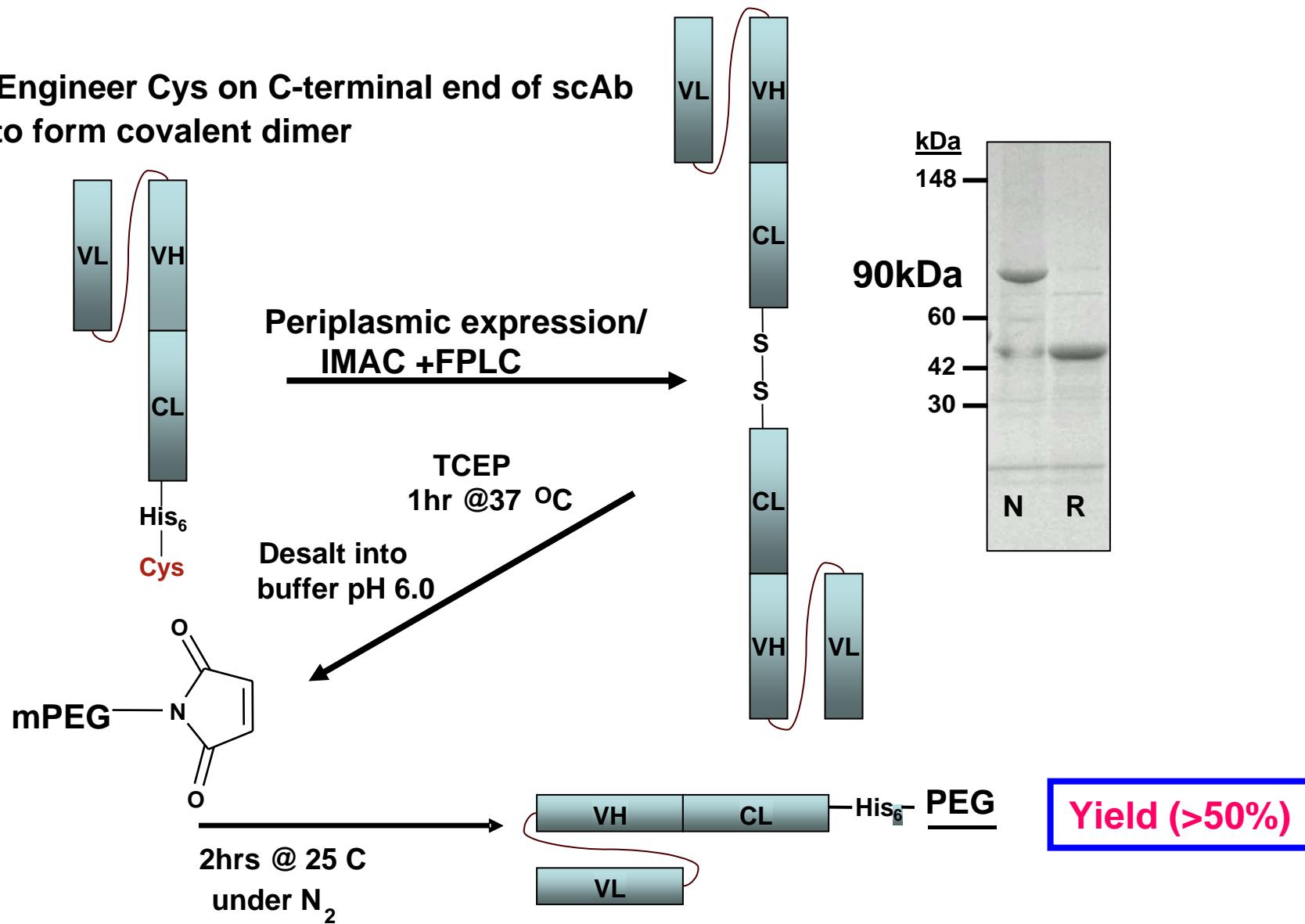
Overlay

Top



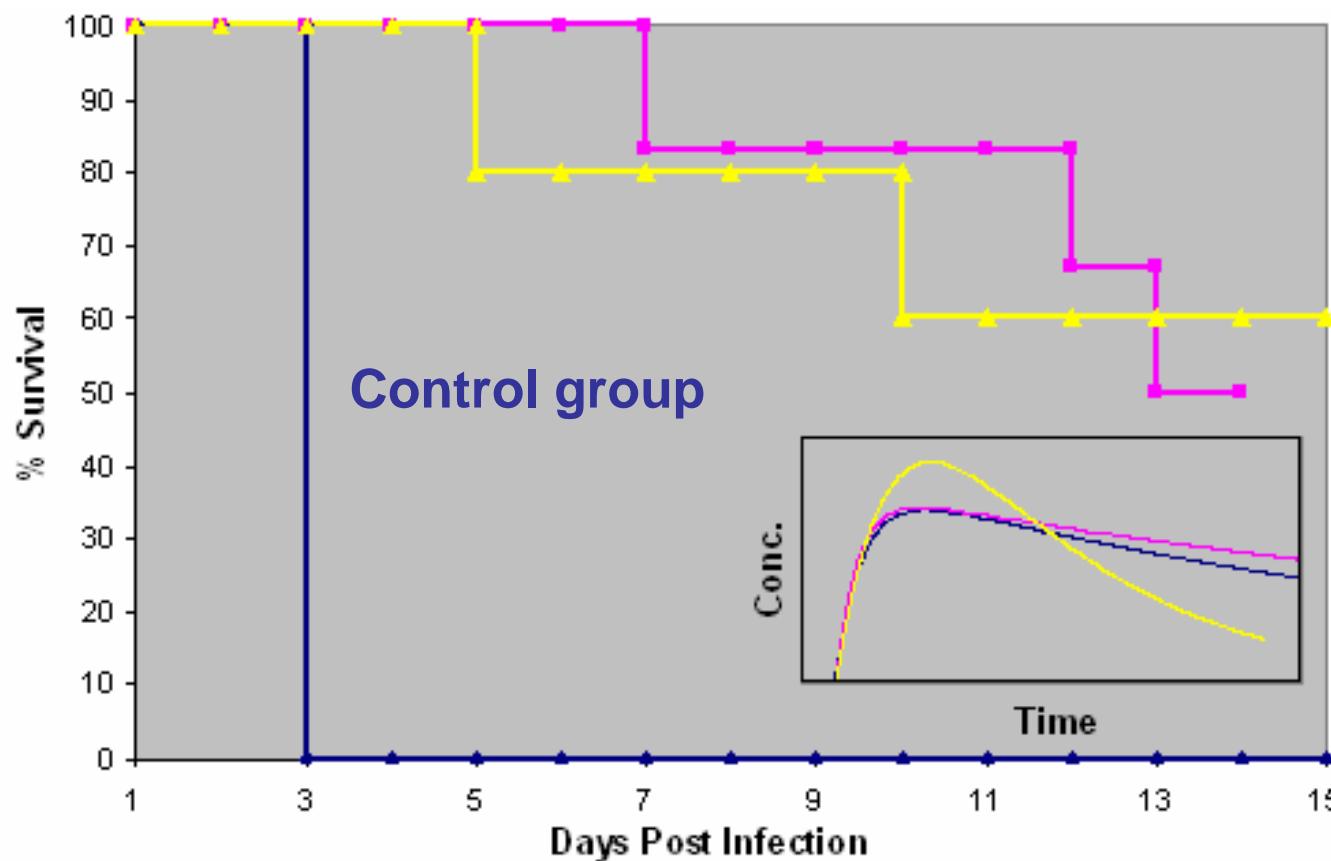
# Preparative ScAb PEGylation

- Engineer Cys on C-terminal end of scAb to form covalent dimer



## Protection Against Challenge w/ Anthrax Spores

- Guinea pig model
- Challenge w/ 500 LD<sub>50</sub> spores



## Passive Protection by Polyclonal Antibodies against *Bacillus anthracis* Infection in Guinea Pigs

S. F. LITTLE,\* B. E. IVINS, P. F. FELLOWS, AND A. M. FRIEDLANDER

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# The detection of protective antigen (PA) associated with spores of *Bacillus anthracis* and the effects of anti-PA antibodies on spore germination and macrophage interactions

C.K. Cote<sup>a</sup>, C.A. Rossi<sup>b</sup>, A.S. Kang<sup>c</sup>, P.R. Morrow<sup>c</sup>, J.S. Lee<sup>d</sup>, S.L. Welkos<sup>a,\*</sup>

<sup>a</sup>United States Army Medical Research Institute of Infectious Disease (USAMRIID), Bacteriology Division, 1425 Porter Street, Fort Detrick, Frederick, MD 21702, USA

<sup>b</sup>USAMRIID, Diagnostic Systems Division, 1425 Porter Street, Fort Detrick, Frederick, MD 21702, USA

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<sup>d</sup>USAMRIID, Virology Division, 1425 Porter Street, Fort Detrick, Frederick, MD 21702, USA

Received 1 November 2004; received in revised form 14 February 2005; accepted 14 February 2005

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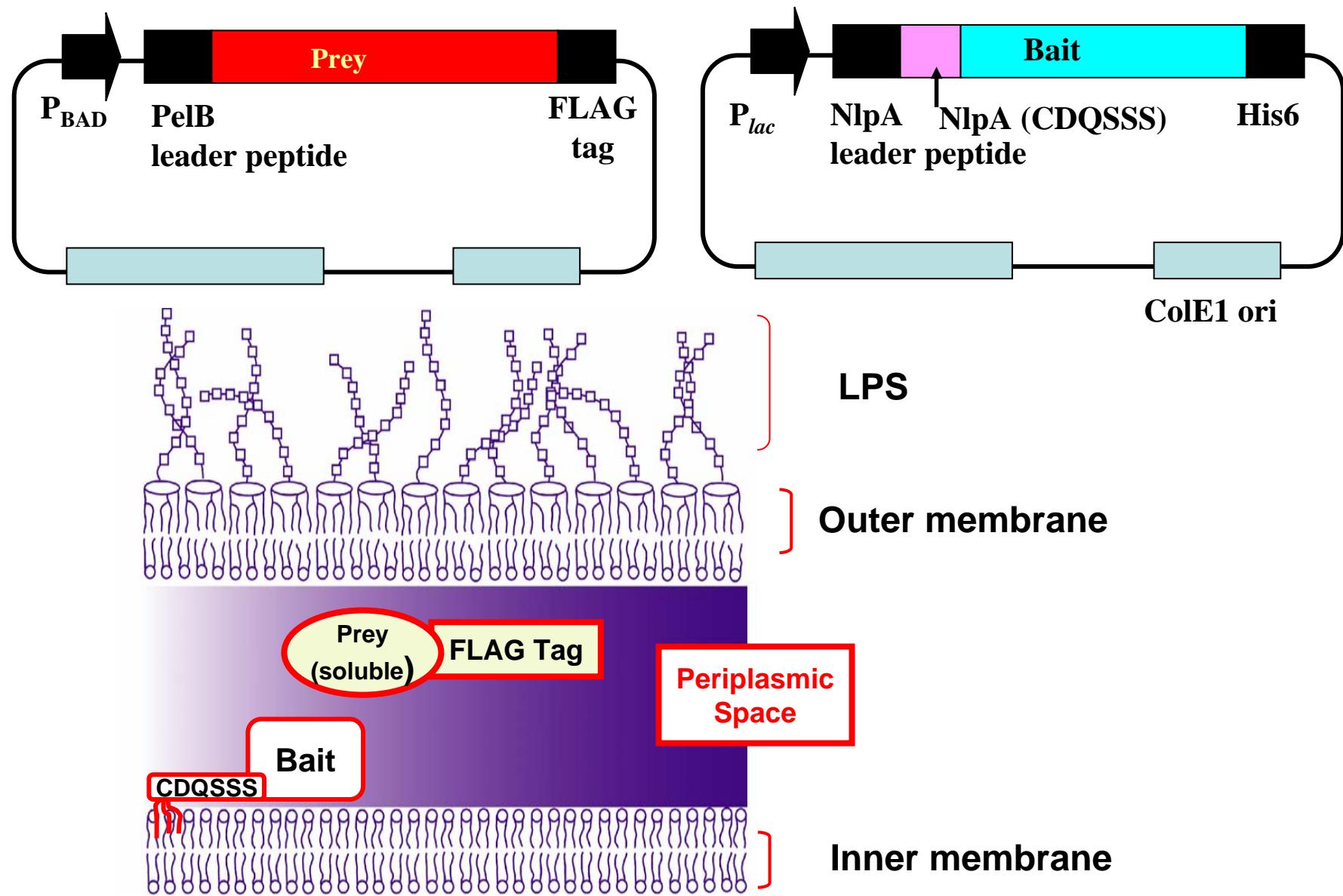
## Abstract

The protective antigen (PA) component of the anthrax toxins is an essential virulence factor of *Bacillus anthracis* and is the major protective immunogen. The kinetics of PA production during growth of *B. anthracis*, and the roles of anti-PA antibody in host immunity are not clearly defined. Production of PA by the vegetative organisms peaks during the shift from exponential to stationary phase of growth. Recently, PA was also found to be associated with spores. In our study, PA-specific mRNA was detected in spores by RT-PCR within 15-min of exposure to germinant. PA protein was detected by immunomagnetic electrochemiluminescence (ECL) on spores within 1 h of exposure to a germination medium and was rapidly released into the supernatant. PA was not demonstrated on ungerminated spores by RNA analysis, ECL, or spore-based anti-PA ELISA; however, it was detected on ungerminated spores by immunoelectron microscopy (immunoem). In rabbits, PA induces polyclonal antibodies (Abs) that, in addition to their anti-toxin neutralizing activities, exhibit anti-spore activities. In this study, the anti-spore effects of a human monoclonal Ab specific for PA (AVP-hPA mAb, Anavir Pharmaceuticals) were characterized. AVP-hPA mAb retarded germination in vitro, and enhanced the phagocytic and sporcidial activities of macrophages. The activities were comparable to those of the polyclonal rabbit anti-rPA Ab. Assays to detect germination inhibitory activity (GIA) in serum from vaccinated mice and guinea pigs suggested a possible role for anti-PA Abs in protection. Thus, anti-PA Ab-mediated, anti-spore activities may play a role in protection during the early stages of an anthrax infection.

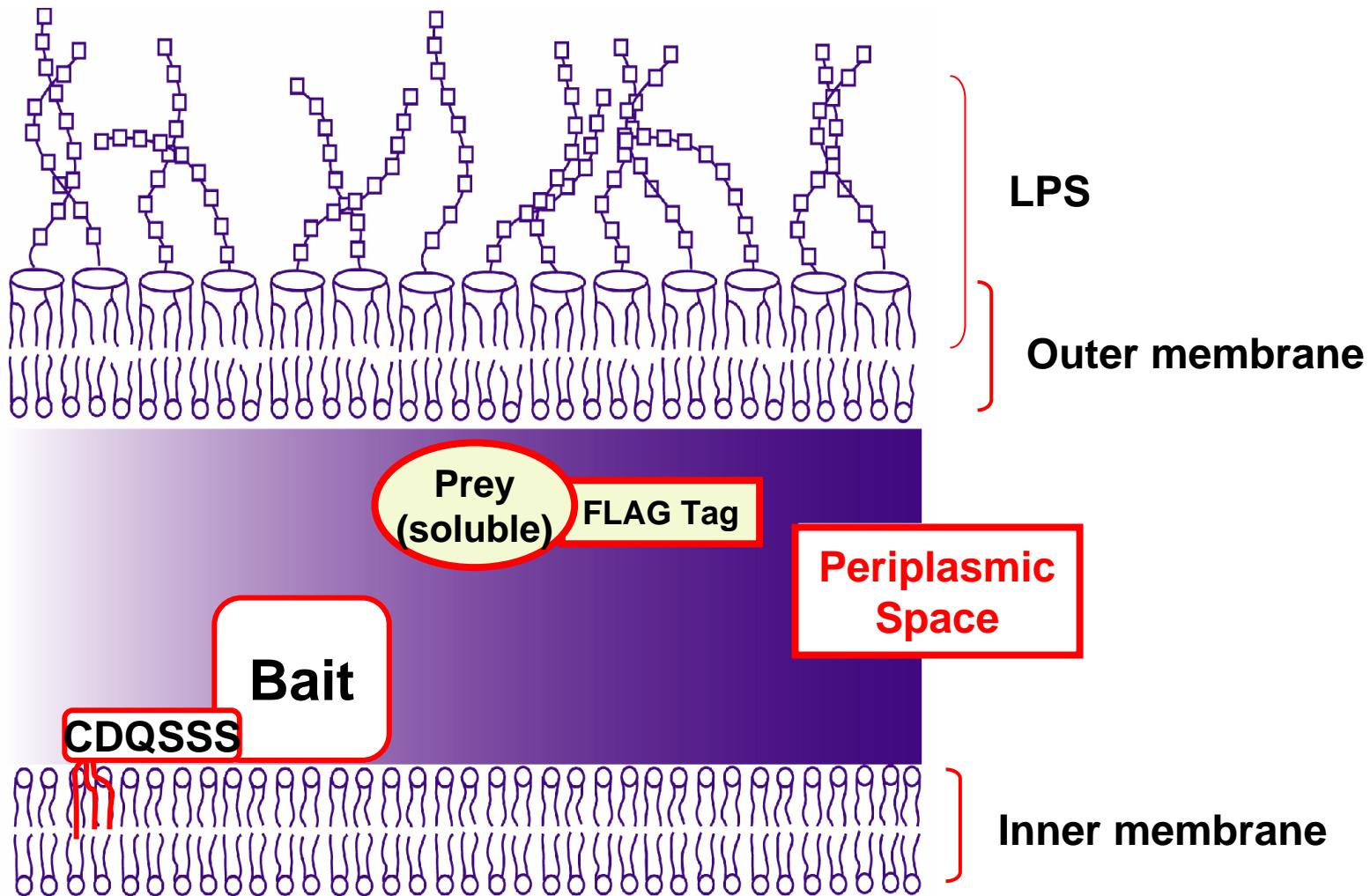
© 2005 Elsevier Ltd. All rights reserved.

**Keywords:** Anthrax; *Bacillus anthracis*; Spores; Protective antigen; Anti-PA antibodies; Immunity

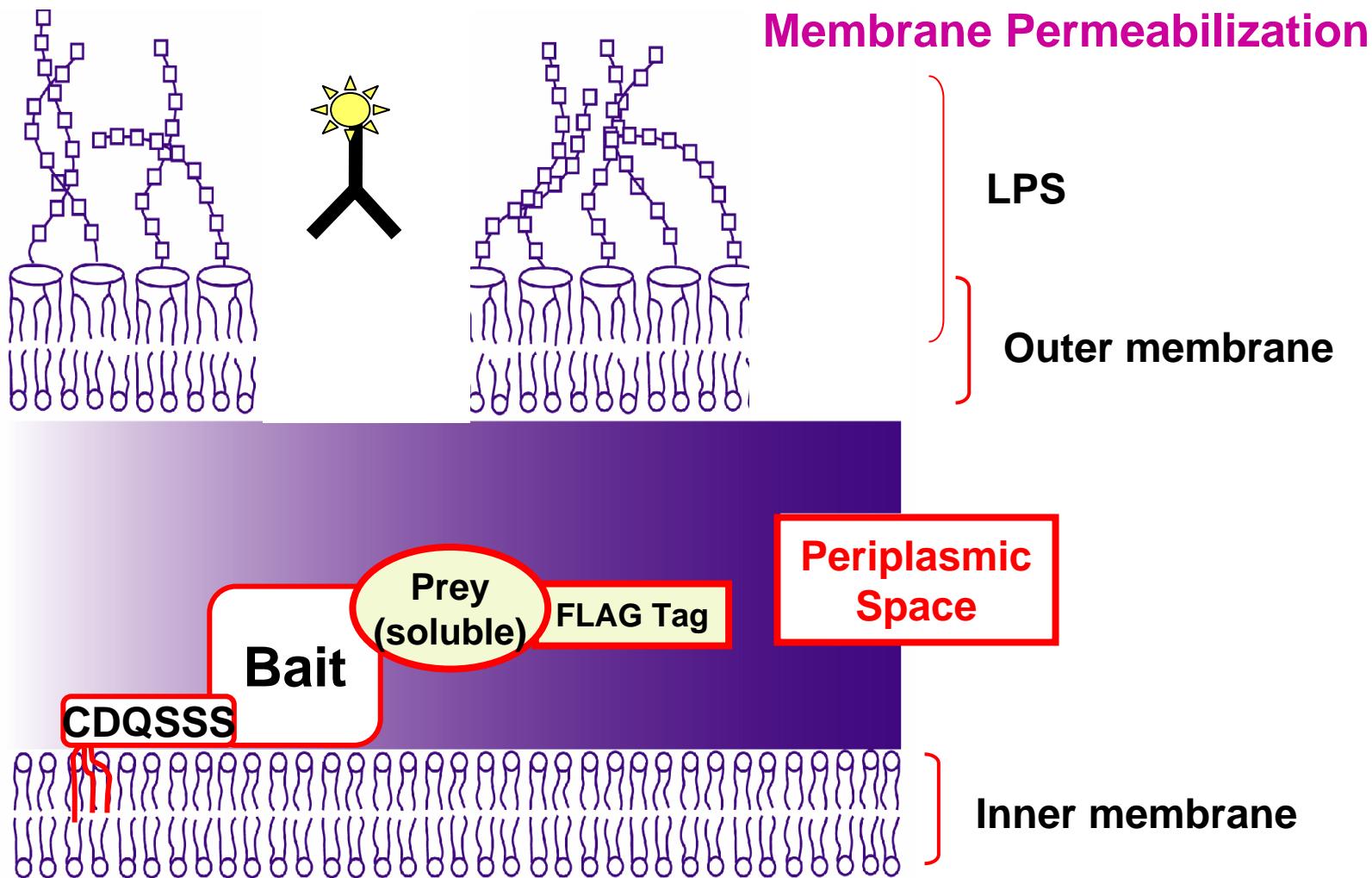
## II. APEx 2-Hybrid Technology (A quantitative high throughput approach)



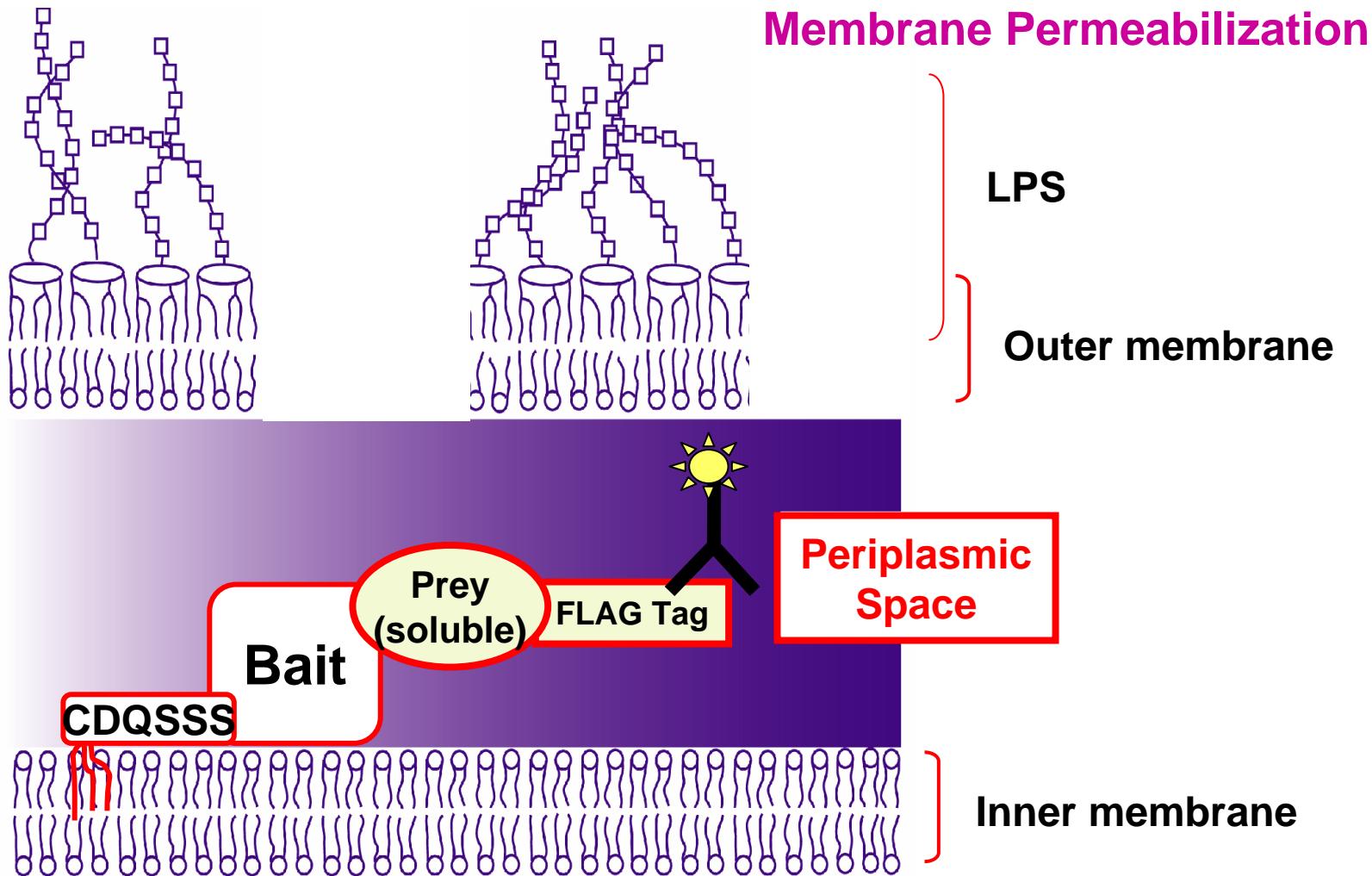
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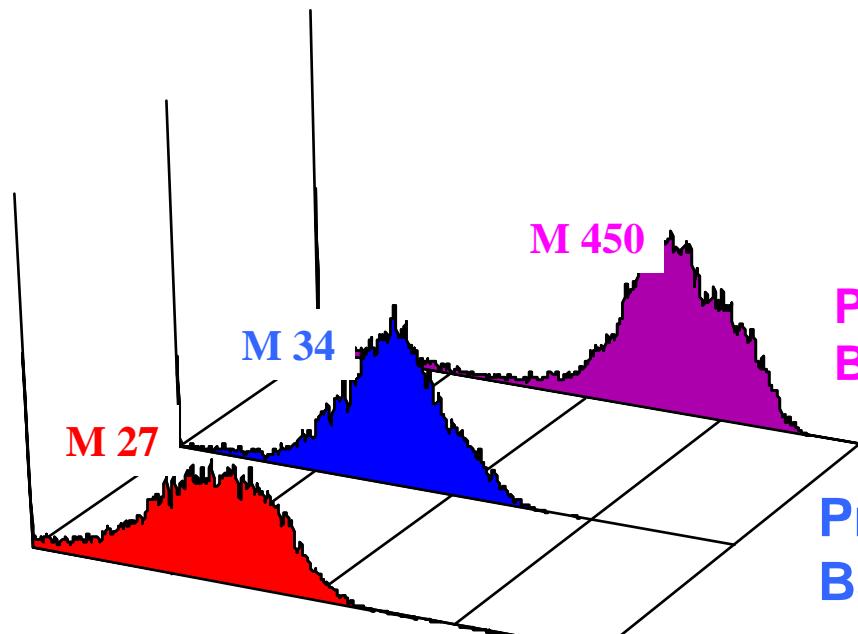


## II. APEx 2-Hybrid Technology (A quantitative high throughput approach)



*Cell fluorescence proportional to Bait:Prey dissociation rate*

## Detecting Protein:Protein Interactions by APEx 2-Hybrid



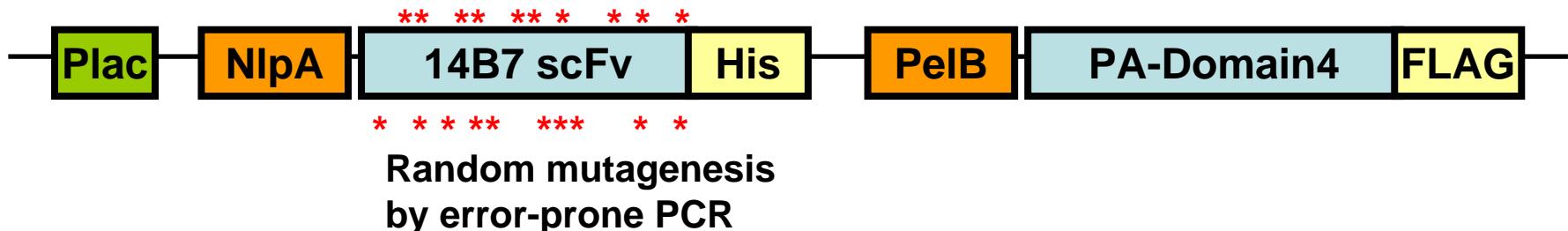
Prey: Antigen (PA-D4)  
Bait: anti-PA scFv

Prey: Antigen (PA-D4)  
Bait: unrelated scFv (26-10 anti-dig)

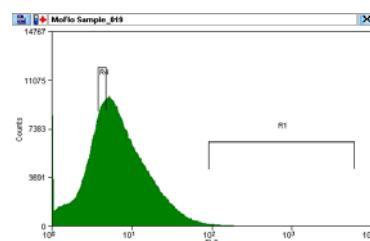
Prey alone: Antigen (PA-D4)

# Isolation of High Affinity Antibodies to Endogenously Expressed Antigen

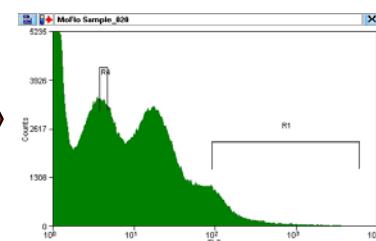
Dicistronic Expression of Ab & Ag Genes in Single Plasmid)



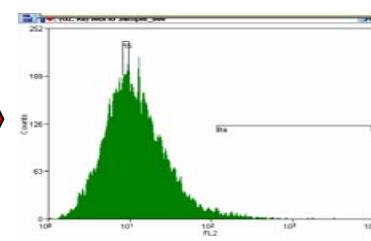
Sorting of positive clones by FACS on a Cytomation MoFlo



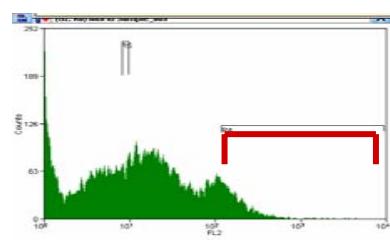
Library  
1st round sorting



1st round  
Re-sorting



2nd round  
sorting

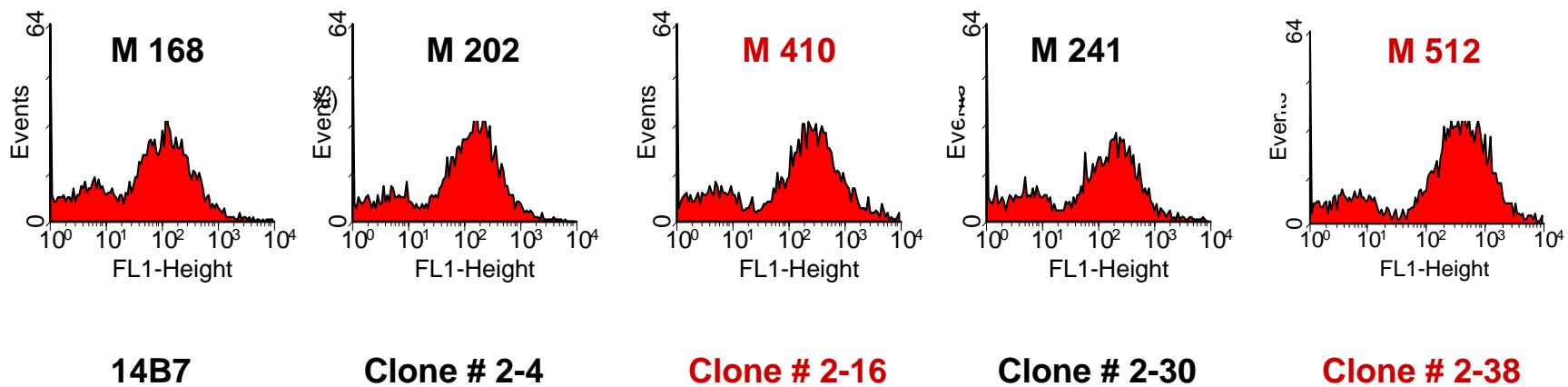


2nd round  
Re-sorting

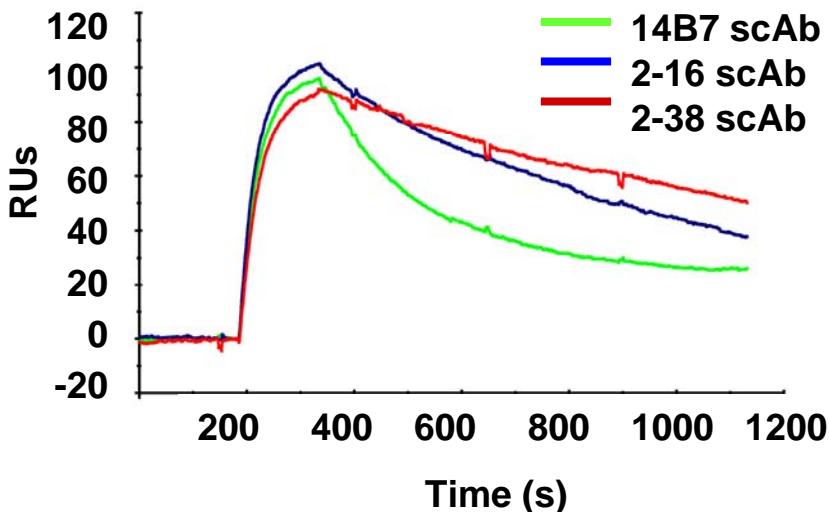


Analyzed individual clones from  
this round for improved affinity

## Individual scanning after 2nd round sorting



### SPR analysis of anti-PA scAb binding to PA



scAb	K <sub>D</sub> (pM)	K <sub>off</sub> (sec <sup>-1</sup> )
14B7	4600	2.8 x 10 <sup>-3</sup>
2-16	480	4.9 x 10 <sup>-4</sup>
2-38	270	2.6 x 10 <sup>-4</sup>

>17 fold increase in affinity after one round of mutagenesis

# Acknowledgements

Professor George Georgiou, Chemical Engineering and Biomedical  
Engineering, University of Texas, Austin

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Steven Pincus  
Giovanni D'Alia  
Linda Nardone  
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Clint Leyseth (UT)  
Jean Patterson (SFBR)  
Ricardo Carrion (SFBR)  
Robert Geiger (SFBR)  
Kathleen Brasky (SFBR)  
Rick Lyons (UNM)  
Stephen Leppla (NIH)

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